UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

June 09, 2015

MEMORANDUM

Subject: Efficacy Review for Clorox WF; EPA File Symbol 5813-RNI; DB Barcode: D425787

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Applicant: The Clorox Company
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Formulation from the Label:

Active Ingredient	% by wt.
Sodium hypochlorite	2.0 %
Other Ingredients:	98.0 %
Total	100.0 %

I. BACKGROUND

The product, Clorox WF (EPA File Symbol 5813-RNI), is a new product. The applicant requested to register the product for use as a disinfectant (bactericide, fungicide, virucide), on hard, non-porous surfaces. Label directions suggest that the product is effective as a disinfectant on visibly clean hard surfaces. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package, identified as D425787, contained letters from the applicant to EPA (dated December 31, 2014); EPA Form 8570-1 (Application for Pesticide); EPA Form 8570-27 (Formulator's Exemption Statement); EPA Form 8570-34 (Certification with Respect to Citation of Data); EPA Form 8570-35 (Data Matrix); seven studies (MRID Nos. 495259-09 through 495259-15), Statements of No Data Confidentiality Claims for all seven studies, and the proposed label (dated 12/9/14).

II. USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: appliance exteriors, bathtubs, countertops, diaper pails, enamel floors, exterior toilet surfaces, floors, glass, high chairs, linoleum, plastic patio furniture, sealed granite, shower doors, showers, sinks, tabletops, toilet handles, and urinals. The proposed label indicates that the product may be used on hard, non-porous surfaces, including: enamel, glass, glazed ceramic, glazed porcelain, glazed tile, Marlite, plastic laminate, synthetic marble, and vinyl. Directions on the proposed label provide the following information regarding use of the product:

To [Clean and] Disinfect Hard Nonporous Surfaces: Pour this product directly on surface. Do not dilute. Use a clean cloth, mop or sponge to wipe surface or spread product. Allow surface to remain wet for 5 minutes. Rinse and air dry. For heavily soiled surfaces, preclean surface. Not for food contact surfaces.

To Kill Mold [and Mildew]: Preclean hard nonporous surface then follow disinfection instructions above.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots at LCL. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be

treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant at LCL must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots at LCL must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. BRIEF DESCRIPTION OF THE DATA

Note: The active ingredient concentration of Batch No. 14SVB1 was reported to be **1.83%** sodium hypochlorite, Batch No. 14SVB2 was reported to be **1.85**% sodium hypochlorite, and Batch No. 14SVB3 was reported to be **1.83**% sodium hypochlorite. All three batches were at or below the active ingredient Lower Certified Limit declared on the Confidential Statement of Formula.

1. MRID 49525909 "AOAC Use-Dilution Method, Test Organism: *Staphylococcus aureus* (ATCC 6538)" for Clorox WF, F2014.0078, by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – September 3, 2014. Project Number A16919.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). Three lots (Lot Nos. 14SVB1, 14SVB2, and 14SVB3) of the product, Clorox WF, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 2013. The product was received as a ready-to-use liquid. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture. For testing conducted 7/7/14 the carriers were dried for 38 minutes at 35-37°C at 56.2% relative humidity; for testing conducted 7/8/14 the carriers were dried for 39 minutes at 35-37°C at 54.5% relative humidity; for testing conducted 7/8/14 the carriers were dried for 40 minutes at 35-37°C at 56.4% relative humidity. Each carrier was placed into 10 mL of test substance for 5 minutes at 20°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth and 0.1% sodium thiosulfate. All subcultures were incubated for 48±2 hours at 35-37°C. Following

incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The average geometric mean for the test microorganism was 6.29 log_{10}/carrier for testing conducted 7/7/14, 6.35 log10/carrier for testing conducted 7/8/14, and 6.43 log_{10}/carrier for testing conducted 7/7/14.

2. MRID 49525910 "AOAC Use-Dilution Method, Test Organism: *Salmonella enterica* (ATCC 10708)" for Clorox WF, F2014.0078, by Kristen Niehaus. Study conducted at ATS Labs. Study completion date – September 3, 2014. Project Number A16921.

This study was conducted against *Salmonella enterica* (ATCC 10708). Three lots (Lot Nos. 14SVB1, 14SVB2, and 14SVB3) of the product, Clorox WF, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 2013. The product was received as a ready-to-use liquid. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture. The carriers were dried for 38 minutes at 35- 37°C at 53.0% relative humidity. Each carrier was placed into 10 mL of test substance for 5 minutes at 19.0-20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth and 0.1% sodium thiosulfate. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The average geometric mean for the test microorganism was 5.76 log_{10}/carrier.

3. MRID 49525911 "Fungicidal Use-Dilution Method, Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)" for Clorox WF, F2014.0078, by Becky Lien. Study conducted at ATS Labs. Study completion date – August 22, 2014. Project Number A16945.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 14SVB1 and 14SVB2) of the product, Clorox WF, were tested using the Fungicidal Use-Dilution Method as described in the AOAC Official Methods of Analysis, 2013. The product was received as a ready-to-use liquid. Ten (10) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture. The carriers were dried for 38 minutes at 35-37°C at 54.6% relative humidity. Each carrier was placed into 10 mL of test substance for 5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.05% Tween 80. After 25-60 minutes, carriers were transferred to secondary neutralization subcultures with 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.05% Tween 80. All neutralized subcultures were incubated for 10 days at 25-30°C, and the agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The average geometric mean for the test microorganism was 4.96 log_{10}/carrier.

4. MRID 49525912 "Fungicidal Use-Dilution Method, Test Organism: *Aspergillus brasiliensis* (ATCC 16404)" for Clorox WF, F2014.0078, by Becky Lien. Study conducted at ATS Labs. Study completion date – August 22, 2014. Project Number A16944.

This study was conducted against *Aspergillus brasiliensis* (ATCC 16404). Two lots (Lot Nos. 14SVB1 and 14SVB2) of the product, Clorox WF, were tested using the Fungicidal Use-Dilution Method as described in the AOAC Official Methods of Analysis, 2013. The product was received as a ready-to-use liquid. Ten (10) stainless steel penicylinder carriers per product lot were inoculated by immersion in a suspension of the organism, at a ratio of one carrier per 1 mL of culture. The carriers were dried for 38 minutes at 35-37°C at 54.6% relative humidity. Each carrier was placed into 10 mL of test substance for 5 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.05% Tween 80. After 25-60 minutes, carriers were transferred to secondary neutralization subcultures with 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.05% Tween 80. All neutralized subcultures were incubated for 10 days at 25-30°C, and the agar plate subcultures were incubated for 44-76 hours at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population. The average geometric mean for the test microorganism was 6.17 $\log_{10}$/carrier.

5. MRID 49525913 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus type 37" for Clorox WF, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 29, 2014. Project Number A16898.

This study was conducted against Rhinovirus type 37 (ATCC VR-1147, Strain 151-1) using MRC-5 cells (human embryonic lung cells; ATCC CCL-171) as the host system. Two lots (Lot Nos. 14SVB1 and 14SVB2) of the product, Clorox WF, were tested according to ATS Labs Protocol No. CX18053014.R37 (copy provided). The product was received as a ready-to-use liquid. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 15.5°C at 50% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 5 minutes at 21.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns, and diluted serially. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

6. MRID 49525914 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus" for Clorox WF, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 29, 2014. Project Number A16897.

This study was conducted against Influenza A virus (ATCC VR-544, Strain Hong Kong) using MDCK cells (canine kidney cells; ATCC CCL-34) as the host system. Two lots (Lot Nos. 14SVB1 and 14SVB2) of the product, Clorox WF, were tested according to ATS Labs Protocol No. CX18053014.FLUA (copy provided). The product was received as a ready-to-use liquid. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were

prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 20.0°C at 40% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 5 minutes at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures then were passed through individual Sephadex columns, and diluted serially. MDCK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

7. MRID 49525915 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Poliovirus type 1" for Clorox WF, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 22, 2014. Project Number A16896.

This study was conducted against Poliovirus type 1 (ATCC VR-1562, Strain Chat) using Vero cells (ATCC CCL-81) as the host system. Three lots (Lot Nos. 14SVB1, 14SVB2, and 14SVB3) of the product, Clorox WF, were tested according to ATS Labs Protocol No. CX18053014.POL (copy provided). The product was received as a ready-to-use liquid. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were dried for 20 minutes at 15.5°C at 55% relative humidity. One replicate per product lot was tested. For each lot of product, separate dried virus films were exposed to 2.00 mL of test substance. The virus films were completely covered with test substance. The treated dish was held covered for 5 minutes at 20.0°C. Just prior to the end of the exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus disinfectant mixtures then were passed through individual Sephadex columns, and diluted serially. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus titer, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

V. RESULTS

MRID Number	Organism	No. Carriers Exhibiting Growth/Total Carriers			Carrier Population (Log_{10}CFU/Carrier)
		Batch 14SVB1	Batch 14SVB2	Batch 14SVB3	
495259-09	*Staphylococcus aureus* (ATCC 6538)	1/60	--	--	6.29
		--	3/60	--	6.35
		--	--	0/60	6.43
495259-10	*Salmonella enterica* (ATCC 10708)	0/60	1/60	0/60	5.76

495259-11	*Trichophyton mentagrophytes* (ATCC 9533)	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	--	4.96
495259-12	*Aspergillus brasiliensis* (ATCC 16404)	1° = 0/10 2° = 0/10	1° = 0/10 2° = 0/10	--	6.17

MRID Number	Organism	Results				Dried Virus Control ($TCID_{50}$/0.1mL)
		Description	Batch 14SVB1	Batch 14SVB2	Batch 14SVB3	
495259-13	Rhinovirus type 37, Strain 151-1 (ATCC VR-1147)	10^{-1} to 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	--	$10^{5.00}$
		$TCID_{50}$/0.1mL	$\leq 10^{0.50}$	$\leq 10^{0.50}$	--	
		TCD_{50}/0.1mL	$10^{1.50}$	$10^{1.50}$	--	
		Log Reduction	$\geq$3.50	$\geq$3.50	--	
495259-14	Influenza A virus, Strain Hong Kong, (ATCC VR-544)	10^{-1} to 10^{-2} dilutions	Cytotoxic	Cytotoxic	--	$10^{7.75}$
		10^{-3} to 10^{-8} dilutions	Complete Inactivation	Complete Inactivation	--	
		$TCID_{50}$/0.1mL	$\leq 10^{2.50}$	$\leq 10^{2.50}$	--	
		TCD_{50}/0.1mL	$10^{2.50}$	$10^{2.50}$	--	
		Log Reduction	$\geq$5.25	$\geq$5.25	--	
495259-15	Poliovirus type 1, Strain Chat, (ATCC VR-1562)	10^{-1} dilution	Cytotoxicity	Cytotoxicity	Cytotoxicity	$10^{6.50}$
		10^{-1} to 10^{-6} dilutions	Complete Inactivation	Complete Inactivation	Complete Inactivation	
		$TCID_{50}$/0.1mL	$\leq 10^{1.50}$	$\leq 10^{1.50}$	$\leq 10^{1.50}$	
		TCD_{50}/0.1mL	$10^{1.50}$	$10^{1.50}$	$10^{1.50}$	
		Log Reduction	$\geq$5.00	$\geq$5.00	$\geq$5.00	

III. CONCLUSION

1. The submitted efficacy data **support** the use of the product, Clorox WF, as a disinfectant with bactericidal activity against the following microorganisms on hard, nonporous surfaces in the presence of a 5% organic soil load for a 5 minute contact time:

MRID 49525909	*Staphylococcus aureus* (ATCC 6538)
MRID 49525910	*Salmonella enterica* (ATCC 10708).

Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

2. The submitted efficacy data **support** the use of the product, Clorox WF, as a disinfectant with fungicidal activity against the following microorganisms on hard, nonporous surfaces for a 5 minute contact time:

MRID 49525911	*Trichophyton mentagrophytes* (ATCC 9533)
MRID 49525912	*Aspergillus brasiliensis* (ATCC 16404)

Killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Viability controls were positive for growth. Sterility controls did not show growth.

3. The submitted efficacy data **support** the use of the product, Clorox WF, as a disinfectant with virucidal activity against the viral strains listed below on hard, non-porous surfaces in the presence of a 5% organic soil load for a 5 minute contact time:

MRID 49525913	Rhinovirus type 37, Strain 151-1 (ATCC VR-1147)
MRID 49525914	Influenza A virus, Strain Hong Kong (ATCC VR-544)
MRID 49525915	Poliovirus type 1, Strain Chat (ATCC VR-1562)

Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

IV. LABEL

1. The proposed label claims are **acceptable** regarding the use of the product, Clorox WF, as a disinfectant with bactericidal activity for use on hard, non-porous surfaces against the following microorganisms when used undiluted in the presence of 5% organic soil, for a 5 minute contact time. These claims **are supported** by the applicant's data:

Staphylococcus aureus (ATCC 6538)
Salmonella enterica (ATCC 10708).

2. The proposed label claims are **acceptable** regarding the use of the product, Clorox, as a disinfectant with fungicidal activity on hard, non-porous surfaces when used undiluted for a 5 minute contact time. These claims **are supported** by the applicant's data:

Aspergillus brasiliensis (ATCC 16404)
Trichophyton mentagrophytes (ATCC 9533)

3. The proposed label claims are **acceptable** regarding the use of the product, Clorox WF, as a disinfectant with virucidal activity for use on hard, non-porous surfaces against the following microorganisms when used undiluted in the presence of 5% organic soil, for a 5 minute contact time. These claims **are supported** by the applicant's data:

Rhinovirus type 37 (ATCC VR-1147)
Influenza A virus (ATCC VR-544)
Poliovirus type 1 (ATCC VR-1562)